extensions of time are hereby petitioned under 37 C.F.R. 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0741.

AMENDMENTS

Please amend the application as follows:

In the Claims:

Please cancel claim 17 without prejudice or disclaimer thereto.

In accordance with 37 C.F.R. § 1.121, please substitute for pending claims 16, 18, 19 and 22 the following rewritten versions of the same claims, as amended.

C1

16. (Amended) An isolated protein comprising a splice variant of human telomerase protein.

18. (Twice Amended) The protein of claim 16, wherein the protein comprises one of the amino acid sequences presented in Figure 11 (SEQ ID Nos. 35, 37, 39, 42, 44, 46, 48, 50, 52-54, 56-58, 60-62, 64-66, 68-70, 72-74, 76-78, 80-82, 84-86), or a variant thereof, wherein said variant has at least 75% amino acid identity with said amino acid sequences presented in Figure 11.

C2

19. (Amended) A fragment of said splice variant of human telomerase protein according to claim 16.

C3

22. (Amended) The fragment according to claim 19, wherein said fragment is from 10 to 100 amino acids long.

In the Drawings:

Under 37 C.F.R. 1.121, attached is a red-lined copy of Figs. 7A, 7B and 10A showing proposed changes to Fig. 7A and Fig. 10A. Upon approval by the Examiner of such changes, Applicants will submit new drawings in compliance with 37 C.F.R. 1.84.

In the Specification:

Please amend the specification as follows:

Please replace the paragraph at page 6, starting at line 19 and ending at line 30 with the following paragraph:

C4

Figure 5 shows the results of amplification of cDNAs synthesized from various tissues. Amplification is performed using primers from the hT1 cDNA sequence and the products are blotted and probed with a radiolabeled oligonucleotide from the hT1 sequence. Amplification is also performed on the same samples with a pair of primers from the β-actin gene as a loading control. a: hT1 cDNA control; b: human genomic DNA control; c: no template control; d: normal colon RNA; e: normal testis RNA; f: normal lymphocyte RNA; g: melanoma RNA (cerebral metastasis); h: melanoma RNA (subcutaneous ankle metastasis); i: melanoma RNA (liver metastasis); j: melanoma RNA (lung metastasis); k: melanoma RNA (auxiliary lymph node metastasis); l: melanoma RNA (skin metastasis); m: breast carcinoma RNA; n: breast carcinoma RNA; o: breast carcinoma RNA; p: breast carcinoma RNA.

Please replace the paragraph at page 17, starting at line 3 and ending at line 22 with the following paragraph:

MW C5

Methods for obtaining fragments are well-known in the art. Portions that are particularly useful within the context of this invention contain the catalytic site, individual RTase motifs, the putative ~~exonic~~ alternative intron/exon sequences (see Figure 10), and the like. Oligonucleotides are generally synthesized by automated fashion; methods and apparatus for synthesis are readily available (*e.g.*, Applied Biosystems Inc, CA). Oligonucleotides may contain non-naturally occurring nucleotides, such as nucleotide analogues, a modified backbone (*e.g.*, peptide backbone), nucleotide derivatives (*e.g.*,

biotinylated nucleotide), and the like. As used herein, oligonucleotides refers to a nucleic acid sequence of at least about 7 nucleotides and generally not longer than about 100 nucleotides. Usually, oligonucleotides are between about 10 and about 50 bases, more often between about 18 and about 35 nucleotides long. Oligonucleotides can be single-stranded or in some cases double-stranded. As used herein, portions of a nucleic acid refer to a polynucleotide that contains less than the entire parental nucleic acid sequence. For example, a portion of telomerase coding sequence contains less than a full-length telomerase sequence. A 'portion' is generally at least about seven nucleotides, and may be as many as 10, 20, 25 or more nucleotides in length. A fragment refers to a polynucleotide molecule of any length and can encompass an oligonucleotide, although more usually, but not to be limiting, the term oligonucleotide is used to denote short polynucleotides and the term fragment is used to denote longer polynucleotides.

C5 cont'd.

Please replace the paragraph at page 17, starting at line 23 and ending at page 19, 7 with the following paragraph:

C6

Oligonucleotides for use as primers for amplification and probes for hybridization screening may be designed based on the DNA sequence of human telomerase presented herein. Oligonucleotide primers for amplification of a full-length cDNA are preferably derived from sequences at the 5' and 3' ends. Primers for amplification of specific regions are chosen to generate products of an easily detectable size. In preferred embodiments, primers are chosen that flank the sequences subject to alternative RNA splicing. In preferred embodiments, one set of primers is chosen such that both the product that spans spliced-in sequence as well as the product that spans spliced-out sequence are suitable sizes to be detected under the same reaction conditions. In other embodiments, two sets of primers are used to detect the alternative spliced RNAs. For example, one set of primers flanks the splice junction in order to detect a spliced-out product. The second set of primers may be derived very close to the junction (such that a spliced-out amplification product is the same size or barely larger than a primer-dimer length) or one or more of the set may be derived from the spliced-in sequence (such that the spliced-out RNA would not yield any product).

Please replace the paragraph at page 19, starting at line 13 and ending at line 22 with the following paragraph:

C7

In addition to the reference telomerase DNA and protein sequences presented in Figures 1, several RNA splice variants are observed. Although some of the variants may reflect incompletely processed mRNA, it is noteworthy that such variants are abundant in an RNA sample (LIM1215) preselected for polyadenylated mRNA. These findings, together with their clustering in the RT domain, suggest that the insertion variants more likely reflect regulation of hT1 protein expression. For example, variants (see α, β Fig. 7) are likely alternative mature coding for variant proteins. Additional evidence in support of alternative proteins comes from sequence analysis of cDNA clones identified in a LIM1215 cDNA library that contained both deletions and insertions compared to the reference sequence.

Please replace the paragraph at page 19, starting at line 23 and ending at line 29 with the following paragraph:

C8

replace F9

At least seven different putative exons appear to be retained in mRNAs (see Figure 7, which displays 6 of the 7 exons). The exons may be independently retained, thus, a particular mRNA may have none, any one, two, etc. up to seven exons. The maximum number of different mRNAs resulting from seven independently spliced exons is 2^7, or 128 different mRNAs. DNA sequences of these exons are presented in Figure 10. The 5' most exons, called sequence "X", is an unknown length, and only a partial sequence is presented.

Please replace the paragraph at page 20, starting at line 1 and ending at line 7 with the following paragraph:

C9

F10

The reference telomerase sequence (Figure 1) includes exon α and exon β. In the following discussion, the effect of presence/absence and location of each exon is presented on the basis that it is

C9 F10 cont- the only alteration. It will be appreciated that a particular exon may alter the sequence of the translated product, regardless of whether other exons are spliced in or out. For example, the presence of exon 1 results in a frameshift and truncated protein, regardless of whether exons α, β, 2 or 3 are spliced in or out.

Please replace the paragraph at page 20, starting at line 8 and ending at line 13 with the following paragraph:

F10 C10 The presence of exon "X" results in a truncated protein that contains approximately 600 N-terminal amino acids and lacks all of the RTase motifs. The presence of exon "Y" at base 222 results in a frameshifted protein that terminates within three codons past the exon. As the Y exon is very GC rich, approximately 78%, which is difficult to sequence, it is possible that exon Y causes an insertion of about 35 amino acids and not a frameshift.

Please replace the paragraph at page 20, starting at line 14 and ending at line 16 with the following paragraph:

C11 F12 Exon 1 at nucleotide 1950 is 38 bp and its presence in mRNA causes a frame-shift and ultimate translation of a truncated protein (stop codon at nt 1973). This truncated protein contains only RTase domains 1 and 2.

Please replace the paragraph at page 20, starting at line 17 and ending at line 22 with the following paragraph:

C12 Exon α, located from bases 2131-2166 is frequently observed spliced out of telomerase mRNA. A protein translated from such an RNA is deleted for 12 amino acids, removing RTase

Sub C3

C12 cont.

motif A. This motif appears to be critical for RT function; a single amino acid mutation within this domain in the yeast EST2 protein results in a protein that functions as a dominant negative and results in cellular senescence and telomere shortening.

Please replace the paragraph at page 21, starting at line 4 and ending at line 13 with the following paragraph:

C13

Exon 2 at base 2843 contains an in-frame termination codon, resulting in a truncated protein that has the entire RTase domain region, but lacks the C-terminus. As the C-terminus may play a regulatory role, protein activity will likely be affected. When exon 3 is retained, a smaller protein is also produced because the exon contains an in-frame stop codon. Thus, the protein has an altered C-terminal sequence. What activity such proteins might have is currently unknown. The crystal structure of the HIV-1 reverse transcriptase demonstrates that a short form of the protein (p51) that lacks the RNAase domain is inhibited by the C-terminal 'connection' folding into the catalytic cleft. If hT1 is assumed to adopt a similar structure to HIV-RT, then C-terminal hT1 protein variants may reflect a similar mechanism of regulation.

Please replace the paragraph at page 21, starting at line 14 and ending at line 24 with the following paragraph:

Sub C5

C14

In addition to variants that lack the reference C-terminal domain, a variant with exon 3 at base 2157 expresses an alternative C-terminal domain. Furthermore, the coding region donated by exon 3 has a potential SH3 binding site, SGQPEMEPPRRPSGCVG, which matches the consensus c-Abl SH3 binding peptide (PXXXXPXXP) found in proteins such as ataxia telangiectasia mutated (ATM). A second example of this motif is found within the N-terminal end of the hT1 protein in the peptide HAGPPSTSRPPRPWDTP. Other alternative C-terminal domains are found in telomerase cDNAs; the EST12462 (GenBank Accession No. AA299878) has about 50 bases of identical sequence up to base 2157 and then diverges from the reference telomerase sequence as well as exon 3. This new sequence has an internal stop codon in 50 bases that would result in a truncated C-terminus.

Please replace the paragraph at page 22, starting at line 1 and ending at line 8 with the following paragraph:

C15

The following table summarizes the splice variants and resulting proteins. For simplicity, only a single variant is listed for each resulting protein. Furthermore, as noted above, the presence of the Y exon appears to cause a frameshift resulting in a truncated protein, but may cause an insertion. Thus, each reading frame of the Y exon is presented and the table is constructed as if the insertion does not cause a truncated protein. An independent assortment of these known exons would lead to 128 different mRNA sequences. The DNA and amino acid sequences for the variants in Table 1 are presented in Figure 11.

Please replace the paragraph at page 26, starting at line 20 and ending at line 29 with the following paragraph:

C16

Peptides of particular interest within the context of this invention have the sequence of the exon sequences (Figure 10), the RTase motifs, and the like. In certain embodiments, telomerase proteins have the amino acid sequences presented in Figures 1 or 11, or a portion thereof which is at least 8 amino acids in length (and may be 10, 15, 20 or more amino acids in length). In other embodiments, the protein has one or more amino acid substitutions, additions, deletions. In yet other embodiments, the protein has an amino acid sequence determined by a nucleic acid sequence that hybridizes under normal stringency conditions to the complement of any of the sequences in Figure 11. As indicated above, variants of telomerase include allelic variants.

Please replace the paragraph at page 30, starting at line 1 and ending at line 15 with the following paragraph:

C17

As discussed above, in preferred embodiments, expression of the various RNA species is monitored. The different species may be assayed by any method which distinguishes one of the species over the others. Thus, length determination by Northern, RNase probe protection, cloning and amplification are some of the available methods. In preferred embodiments, RNase probe

C17 cont.

protection and amplification are used. For RNase probe protection, the probe will generally be a fragment derived from the junction of the reference sequence and the exon sequence or derived from the sequence surrounding the exon insertion site. For example, a fragment of the reference telomerase that spans nucleotide 1950-1951 (*e.g.*, nucleotides 1910-1980) will protect the reference sequence as a 71 base fragment, but will protect a telomerase with exon 1 as two fragments of 41 and 30 bases. In contrast, a fragment that contains nucleotides 1910-1950 and 30 bases of exon 1 will protect an exon 1 variant as a 71 base fragment and the reference telomerase as a 41 base fragment. Fragments for RNase probe protection are chosen usually in the range of 30 to 400 bases and are positioned to yield readily distinguishable protection products.

Please replace the paragraph at page 37, starting at line 1 and ending at line 17 with the following paragraph:

C18

Another inhibitor of the present invention is antisense RNA or DNA to telomerase coding or non-coding sequence. Antisense nucleic acids directed to a particular mRNA molecule have been shown to inhibit protein expression of the encoded protein Based upon the telomerase sequences presented herein, an antisense sequence is designed and preferably inserted into a vector suitable for transfection into host cells and expression of the antisense. The antisense may bind to any part of the hTI RNA. In certain embodiments, the antisense is designed to bind specifically to one or more variants. Specific binding means that under physiological conditions, the antisense binds to RNAs that have the complementary sequence, but not other RNAs. Because telomerase RNAs that contain any particular exon sequence may be a heterogeneous group of variants due to independent assortment of splice variants, more than one species of RNA may be bound and inactivated. The antisense polynucleotides herein are at least 7 nucleotides long and generally not longer than 100 to 200 bases, and are more typically at least 10 to 50 bases long. Considerations for design of antisense molecules and means for introduction into cells are found in U.S. Patent Nos. 5,681,747; 5,734,033; 5,767,102; 5,756,476; 5,749,847; 5,747,470; 5,744,362; 5,716,846).

Please replace the paragraph at page 39, starting at line 26 and ending at page 40, line 6 with the following paragraph:

C19

Therefore, the exon sequences may be especially useful for diagnostic applications. For example, detection and identification of diseases, such as cancer, aging, wound healing, neuronal regeneration, regenerative cells (*e.g.*, stem cells), may be important preludes to determining effective therapy. In this regard, detection of wound healing can facilitate development and identification of an ameliorative compound. Currently, wound healing assays are expensive and time consuming, whereas an amplification or hybridization-based assay would be quick and cost effective. In any of these applications, detection may be quantitative or qualitative. In a qualitative assay, a particular amplification primer pair or hybridization probe for one of the variant sequences (*e.g.*, exons that are variably spliced) can be used to detect the presence or absence of the variant sequence.

Please replace the paragraph at page 40, starting at line 29 and ending at page 41, line 4 with the following paragraph:

C20

For amplification assays, primer pairs that either flank the exons or require the presence of the exon for amplification are desirable. Many such primer pairs are disclosed herein. Others may be designed from the sequences presented herein. Generally, the primer pairs are designed to only allow amplification of a single exon, however, in some circumstances detection of multiple exons in the same RNA preparation may be preferred.

Please replace the paragraph at page 55, starting with line 21 and ending at page 56, line 3 with the following paragraph:

C21

To test the hypothesis that such a transcript exists, a primer, HTM2028F, is designed such that amplification ensues only when the 36 bp fragment was missing. Amplification using HTM2028F and HT2026F primers in combination with HT2356R demonstrate that transcripts containing the 182 bp fragment but missing the 36 bp fragment are present in LIM1215 RNA (Figure 9, lanes a and b). The same top strand primers (HTM2028F and HT2026F) in combination with HT2482R primer amplify a number of products from LIM1215 RNA (Figure 9, lanes c and d), most

of which represent bands 1- 4 as determined by direct sequence analysis of PCR products. An amplified fragment of 650 bp using HTM2028F and HT2482R primers represents another, not yet fully characterized, alternatively spliced telomerase variant in the RT-MotifA/RT Motif B region. For clarity of presentation, the protein sequence giving the best match with *Euplotes* and *S. cerevisiae* proteins is presented in Figure 2 as the reference sequence.

Please replace the paragraph at page 56, starting at line 4 and ending at line 24 with the following paragraph:

Specifically, there are at least seven inserts or exons that can be present (or absent) from telomerase RNA. (1) The 5'-most sequence (Y) is located between bases 222 and 223. (2) the insert (X) is located between bases 1766 and 1767. A partial sequence is determined and is presented in Figure 10. Termination codons are present in all three reading frames. Thus, a truncated protein without any of the Rtase motifs would be produced. (2) A sequence, indicated as "1" in Figure 7, is located between bases 1950 and 1951. This exonic sequence is 38 bp (Figure 10) and appears to be present in ALT and most tumor lines. The presence of this sequence adds 13 amino acids and shifts the reading frame, such that a termination codon (TGA) is in frame at nucleotide 1973. (3) A sequence, indicated as "α" in Figure 7, is located between bases 2130 and 2167. This sequence is 36 bp (Figure 10) and its absence removes RTase motif "A" but does not alter the reading frame. (4) A sequence, indicated as "β" in Figure 7 is present between bases 2286 and 2469. The insert is 182 bases (Figure 10) and its absence causes a reading frame-shift and a termination codon in RTase motif 5 at nucleotide 2604. (5) The sequence "2" in Figure 7 is present between bases 2823 and 2824. Its length is undetermined; its partial sequence is presented in Figure 10. The presence of this insert causes a truncated telomerase protein, as the first codon of the insert is a termination codon. (6) The sequence "3" is a 159 bp insert (Figure 10) between bases 3157 and 3158. Its presence leads to a telomerase protein with an altered COOH-terminus. The insert contains a stop codon. Moreover, sequence "3" has a putative binding site for the SH3 domain of c-*abl* (PXXXXPXXP; PEMEPPRRP).